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Studies on Some Characters of *Corticium centrifugum* Parasitic on Calabash Plant

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Introduction

In September, 1930, the writer's notice was attracted by the serious disease of calabash (*Lagenaria vulgaris* var. *depressa*) in the vicinity of Utsunomiya. After studying its cause carefully, it was decided that the disease was caused by *Corticium centrifugum* (Lév.) Bres. This is a case of Shirakinu disease.

The writer isolated the causal fungus on artificial media and made studies of some of its morphological, physiological as well as pathogenic characters.

Historical Review

The causal fungus of the present disease was first described by Rolfs (7) in 1893. In 1911, Saccardo (8) reported regarding the same fungus under the name *Sclerotium Rolfsii* Sacc. studying a specimen sent to him by Stevens.

In 1910, Sawada (9) described *Sclerotium* disease of melons caused by *Hypochnus Cucumeris* Fr. Further, he (10) found a spore form of sclerotium on camphor-trees and decided the fungus to be *Hypochnus centrifugus* (Lév.) Tul. He recognized as its synonyms *Hypochnus Cucumeris* Fr., *Hypochnus Solani* Prill. et Delac., *Hypochnus Theae* Barnard., *Sclerotium Castaneum* Speg.,

Corticium centrifugum (Lév.) Bres., (11) *Sclerotium Rolfsii* Sacc. and *Rhizoctonia destruens* Tass.²

Nakata (4, 5) found a spore form on infected sugar beets and elephant foot plants (*Amorphophallus Konjac*) inoculated with three different strains of *Sclerotium Rolfsii* Sacc. and its mutants, and he recognized this spore form to be *Hypochnus centrifugus* (Lév.) Tul.

In 1930, Goto (1) found some spore forms of *Sclerotium Rolfsii* Sacc. on culture media and changed the name *Hypochnus centrifugus* (Lév.) Tul. to *Corticium centrifugum* (Lév.) Bres., in view of the recent tendency of the classification of Thelephoraceae.

Symptoms of the Disease

This disease of calabash appears in the field in late summer, in ordinary years about the middle of August, sometimes two or three weeks earlier or later.

The mycelial mass, generally with white silky-lustre appears at first on the stem contact with the ground. The surface of the affected areas assumes a brown, water-soaked appearance irregularly shaped, and later becomes slightly sunken, and gum is excreted there. The upper parts of the affected stems often wilt. Further, affected areas undergo conspicuous change in colour, and are deeply sunken and rotten. In fine weather such parts dry up.

As the disease progresses, the entire plant wilts and die. At last numerous sclerotia of millet grain shape are produced, which at first are white in colour, next change to light-brown, and finally become yellowish brown.

Morphology of the Causal Fungus

In the following paragraphs describing the morphological characters of the causal fungus, the colours are shown according to Ridgway's Color Standard and Color Nomenclature (6).

On host.

1. Hyphae

The young hyphae are generally from pure white to white, but when old, "Pale Olive-Buff." Hyphae septate and branch, sometimes contain

numerous granules and vacuoles, and rarely produce clamp connections; hyphae measure $2.50-6.25\ \mu$ ($5\ \mu$ common) in width.

2. Sclerotia

Sclerotia are produced abundantly on the surface of affected areas. They are generally spherical, ellipsoidal and irregular, measuring $0.58-1.19 \times 0.51-0.99\ \text{mm}$ ($0.66 \times 0.83\ \text{mm}$ common) and have a very short stalk.

The colour of sclerotia is "Vinaceous-Buff" or "Vinaceous-Fawn."

On culture media.

1. Hyphae

Hyphae are generally from pure white to white, but those produced on soy agar after Saito's formula and apricot decoction agar present "Pale Vinaceous-Fawn", "Pale Cinnamon-Pink", or "Pale Pinkish Buff." Hyphae septate, branch, sometimes contain numerous granules and vacuoles, and rarely form clamp connections. The width of hyphae is $2.5-10.0\ \mu$ ($3.7\ \mu$ common), and the immersed hyphae are slightly broader than the aerial ones.

2. Sclerotia

The colours of mature sclerotia produced on various culture media are; "Carob Brown", "Deep Olive", "Olive-Brown", "Burnt Umber", "Lincoln Green", "Dark Grayish Olive", "Bone Brown", "Hessian Brown", "Dusky Yellowish Green" and "Dusky Olive-Green."

Their shapes are ellipsoidal, ovoidal, spherical, irregular or connected, and on the surface smooth or slightly sunken.

The size of sclerotia grown on calabash decoction agar in Erlenmeyer flasks for 25 days at 24°C is $1.40-2.70 \times 0.92-1.82\ \text{mm}$ ($1.30 \times 1.40\ \text{mm}$ common). They are 1.7 times in length and 2 times in width compared with sclerotia on host.

The sections through the sclerotia show that the outer layer consists of one or two layers of ellipsoidal, round, polygonal and irregular shaped cells with yellowish brown colour. The middle layer consists of three or four layers of parenchymatous cells which are hexagonal or polygonal in shape and hyaline or light brown in colour. In these cells granules and oil drops refracting light are contained. The inner layer is interwoven with round, ellipsoidal, dumb-bell shaped, long mycelia-like hyaline cells, sometimes containing granules and oil drops.

Cultural Characteristics of the Causal Fungus

The causal fungus was isolated in the ordinary way from other fungi on infected calabash plants in the field. In order to determine the cultural characteristics, the following twenty different media were used as the source of nutrition for the fungus.

A. Nutrient media

Calabash decoction

Fresh stem 200 gr, cane sugar 10 gr, dist. water 1000 cc.

Japanese soy (after Miyoshi's formula)

Onion decoction 250 cc, Japanese soy 200 cc, cane sugar 50 gr, dist. water 500 cc.

Japanese soy (after Saito's formula)

Onion decoction 100 cc, Japanese soy 50 cc, cane sugar 50 gr, dist. water 850 cc.

Apricot decoction

Apricot 150 gr, dist. water 1000 cc.

Potato decoction

Potato 200 gr, cane sugar 20 gr, dist. water 1000 cc.

Rice straw decoction

Dried rice straw 50 gr, dist. water 1000 cc.

Carrot decoction

Carrot 200 gr, glucose 20 gr, dist. water 1000 cc.

Soil extract

Dried soil 70 gr, cane sugar 20 gr, dist. water 1000 cc.

Asparagin synthetic

Asparagin 5 gr, potassium biphosphate 5 gr, cane sugar 50 gr, magnesium sulphate 2.5 gr, iron chloride solution (1%) trace, dist. water 1000 cc.

Richard's solution

Potassium nitrate 10 gr, potassium phosphate 5 gr, magnesium sulphate 2.5 gr, cane sugar 50 gr, dist. water 1000 cc.

B. Agar media

These were made by adding 1.7 % agar to each of the above described nutrient media except apricot decoction to which 3.5 % agar was added.

In each medium the causal fungus was grown in 5 Erlenmyer flasks for 4 weeks at about 24°C. The results of observation made on them are

TABLE 1 Cultural characteristics shown on different nutrient media

Culture media	Mycelia		Sclerotia			
	Growth	Colour	Formation	Colour	Number	Weight (in gr) Weight per one (in gr)
Calabash decoction ¹⁾	++++	"White"	++	"Hessian Brown"	170	0.0018
Japanese soy (after Miyoshi's formula) ²⁾	—	—	—	—	—	—
Japanese soy (after Saito's formula) ³⁾	++++	"Pale Cinnamon Pink"	++++	"Deep Olive"	483	0.0033
Apricot decoction ⁴⁾	++++	"Pale Pinkish Buff"	++++ ++++	"Dark Grayish Olive"	942	0.0019
Potato decoction ⁵⁾	++++	"White"	+++	"Bone Brown"	307	0.0024
Rice straw decoction ⁶⁾	+	do	+	"Lincoln Green"	10	0.001
Carrot decoction ⁷⁾	++++	do	+++	"Dusky Yellowish Green"	270	0.0027
Soil extract ⁸⁾	+	do	+	do	1	0.001
Asparagin synthetic ⁹⁾	+	do	—	—	—	—
Richard's solution	+	do	—	—	—	—

1). Growth of aerial mycelia good, and regular shaped small sclerotia were produced only on the wall of flasks. 2). Aerial mycelia vigorous, and regular shaped large sclerotia were produced. 3). Growth of aerial mycelia good, and regular shaped small sclerotia were abundantly produced on the wall and bottom of flasks. 4). Growth of mycelia good. 5). Growth of mycelia very poor. 6). Growth of mycelia good. 7). Growth of mycelia very poor. 8). do. 9). do.

TABLE 2 *Cultural characteristics shown on different agar media*

Culture media	Mycelia		Sclerotia			
	Growth	Colour	Formation	Colour	Number	Weight (in gr) Weight per one (in gr)
1) Calabash decoction agar	+++	"White"	++	"Carob Brown"	148	0.0018
2) Japanese soy agar (after Miyoshi's formula)	+++++	do	+++	"Deep Olive"	315	0.0042
3) Japanese soy agar (after Saito's formula)	+++++	"Pale Vinaceous-Fawn"	+++++	"Olive-Brown"	790	0.0024
4) Apricot decoction agar	+++++	"White"	+++++	"Burnt Umber"	446	0.0029
5) Potato decoction agar	++	do	+++	"Deep Olive"	311	0.0023
6) Rice straw decoction agar	+	do	+	"Olive-Brown"	20	0.0015
7) Carrot decoction agar	+++	do	+++	"Dusky Olive-Green"	338	0.0025
8) Soil extract agar	++	do	+	do	37	0.0029
9) Asparagin synthetic	++	do	-	-	-	-
10) Richard's solution	++	do	-	-	-	-

1). Growth of mycelia good, and regular shaped sclerotia were produced on the bottom of flasks. 2). Aerial mycelia vigorous, and large, irregular shaped flattened sclerotia were produced on the wall of flasks. 3). Aerial mycelia good, and regular shaped sclerotia of median size were produced on the bottom and wall of flasks. 4). do. 5). Aerial mycelia poor, and regular shaped sclerotia of median size were produced on the bottom and wall of flasks. 6). Growth of mycelia very poor. 7). Growth of mycelia poor, and regular shaped sclerotia of median size were produced. 8). Growth of mycelia poor, and regular shaped sclerotia of median size were produced only on the bottom of flasks. 9). Growth of mycelia poor. 10). do.

summarized in Tables 1 and 2. The number and weight of sclerotia were measured in the material from one flask, in which the growth of fungus was moderate. The number of + shows the grade of growth of fungus and — means no growth at all.

As these Tables show, the growth of mycelia and the formation of sclerotia were most prominent in the nutrient and agar of soy and apricot. Carrot, potato and calabash were next to them in these respects, rice straw and soil extract poorer, asparagin synthetic and Richard's solution very poor.

But a characteristic mycelial layer was produced on agar media of asparagin synthetic. On soy agar after Miyoshi's formula, large irregular shaped and flattened sclerotia were formed, but on the same nutrient media, mycelia were not developed.

The modes of growth of mycelia as well as of the formation of sclerotia were generally more or less different between nutrient and agar media.

The Effect of Temperature on the Growth of the Causal Fungus in Culture

The effect of temperature on the growth of the causal fungus was studied by growing the mycelia on Petri dishes of apricot decoction agar, soy agar and carrot decoction agar.

For each medium and the classes of temperature which are shown in Tables 3 and 4, 5 clean sterilized Petri dishes were prepared and to each of them 10 cc of media were poured.

One of the sclerotia grown on calabash decoction agar or a piece (5 mm in dia.) of mycelial layer on carrot decoction agar was transferred to the center of each dish, and incubated at different temperatures. The rate of their growth was determined by measuring the diameter of colonies produced during the period of 3 days.

The numbers given in Tables 3 and 4 present the average diameter in 5 dishes in centimeter. * in the Tables shows no growth and — no experiment.

As these Tables show, the minimum temperatures for the growth of mycelia from sclerotia and that of mycelia from mycelia of the causal fungus lie between 11° and 15°C. The optimum temperature is about 28°C with the exception in experiments III in Table 4, and the maximum seems to be above 38°C.

TABLE 4 *Effect of temperature on the growth of mycelia from mycelia*

Culture media	No. of exp.	Temperature (°C)						
		6-11	15	20	24	28	32	36
Apricot decoction agar	I	* 5.0	12.6	25.4	36.0	71.8	43.0	14.0
	II	* 5.0	12.8	44.0	70.0	84.0	28.6	—
	III	* 5.0	7.4	41.0	58.0	83.2	80.0	57.6
	Average	* 5.0	10.9	36.8	54.7	79.7	50.5	35.8
	Order of growth	VIII	VI	IV	II	I	III	V
Japanese soy agar (after Saito's formula)	I	* 5.0	12.2	34.8	53.4	81.0	29.4	19.6
	II	* 5.0	11.2	35.4	62.4	77.6	44.6	—
	III	* 5.0	6.2	28.0	48.4	69.0	79.8	64.6
	Average	* 5.0	9.9	32.7	54.7	75.9	51.3	42.1
	Order of growth	VIII	VI	V	II	I	III	IV
Carrot decoction agar	I	* 5.0	12.2	37.8	55.8	82.8	31.0	11.6
	II	* 5.0	12.2	41.0	73.2	84.0	24.0	—
	III	* 5.0	8.0	37.0	62.4	80.0	82.9	65.0
	Average	* 5.0	10.8	38.6	63.8	82.3	45.7	38.3
	Order of growth	VIII	VI	IV	II	I	III	V

Thus the result obtained by the writer approximately agrees with that of Hemmi and Yokogi (2) obtained for the same fungus on sesame, and when compared with Higgins (3), there is a small difference. According to him, the minimum temperature for the mycelial growth of the same fungus on pepper plants is near 8°C, the maximum near 40°C, and the optimum between 30° and 35°C.

Inoculation Experiment

In 1910, Sawada (9) reported that calabash was affected by the fungus under consideration (*Hypochnus Cucumeris* F.) in the field, and the inoculation experiment was carried out with success on the host plant as well as on *Lactuca* sp. *Cryptomeria*, *Abutilon*, squash, egg-plant, etc.

In order to determine the pathogenicity of this fungus the following experiments were made.

A. On host plants

In experiment I, a seedling of calabash was planted on March 16, 1931, in each of a number of unglazed pots (30 cm in dia.) containing fertile soil. In experiments II and III, 5 seeds of calabash were sown on May 13 and July 18 respectively in each pot (20 cm in dia.), and afterwards thinned to 2 plants per pot.

The base of petiole and the internode several centimeters above the ground were sterilized with 80 per cent alcohol, thoroughly washed with sterilized distilled water, and there a puncture was made with a sterilized needle.

In each of the punctures a piece of mycelial layer or one of the sclerotia was inoculated. The same inoculation was made on the normal surface of other plants at the corresponding parts.

The plants thus treated were placed in the thermostat designed by the Phytopathological Laboratory of the Kyoto Imperial University which was saturated with moisture and adjusted at about 28°C. After 2 days, they were removed from the thermostat and observed in out of doors. The results are given in Table 5.

TABLE 5 *The results of inoculation with sclerotia or mycelia on the seedlings of calabash*
A, No. of plants inoculated. B, No. of plants infected. C, % of plants infected.

No. of exp.	Seedlings	Date of inoculation	On normal surface			In punctures		
			With sclerotia	With mycelia	Control	With sclerotia	With mycelia	Control
I	Length of plants 18-23 cm	May 28	4	4	4	4	4	4
	Number of leaves 5-7		4	4	0	4	4	0
			100.0	100.0	0.0	100.0	100.0	0.0
II	Length of plants 10-12 cm	July 1	6	6	6	6	6	6
	Number of leaves 3-4		2	1	0	4	2	0
			33.3	16.7	0.0	66.7	33.3	0.0
III	Length of plants 12-20 cm	Aug. 16	14	14	14	14	14	14
	Number of leaves 3-4		10	6	0	13	5	0
			71.4	42.9	0.0	92.9	35.7	0.0

In experiment I, a reddish brown, ellipsoidal lesion was produced 2 days after the inoculation at the center of the affected areas and later the epidermis was split there. In 2 weeks affected areas became deeply sunken and enlarged, and numerous sclerotia were formed in 5 weeks.

In experiments II and III, the affected areas produced, 2 days after the inoculation, water-soaked lesions with light brown colour and became conspicuously sunken, the epidermis was split there and gum was excreted. Within 2 weeks the affected areas began to be broken, died completely and numerous sclerotia were formed there.

All the control plants remained healthy in experiments I, II and III.

Using plants with punctures or sclerotia the inoculation was more successful than using plants without punctures or mycelia.

B. On rice plants

On Aug. 5, 1931, the seedlings of upland rice (Esoshimamochi) were planted in unglazed pots (30 cm in dia.) containing fertile soil, and the seedlings of paddy rice (Aikoku) in glazed pots (16 cm in dia.) with water and soil in paddy state.

On the inner surface of the base of ligula of the first and second leaves counted from below, and on the outer surface of the blade sheath of the first leaf at the position opposite to its splitting, the inoculation was made with the sclerotia or mycelia. The method of inoculation was the same as in A. Its results are given in Tables 6 and 7.

It seems in these two Tables that the upland rice under consideration is more susceptible to the disease than the paddy rice used.

C. On other plants

On June 5, 1931, the seedlings of pepper, egg-plant, tomato and sweet potato growing vigorously were planted in unglazed pots (15-20 cm in dia.) containing fertile soil and on May 2, 1931, the seeds of various plants presented in Table 8, excepting those above stated, were sown in the analogous pots.

The inoculation was carried out in the same manner as in A. The results are given in Table 8.

TABLE 6 *The results of inoculation with sclerotia or mycelia on upland rice*
A, No. of plants inoculated. B, No. of plants infected. C, % of plants infected.

No. of exp.	Plants	Date of inoculation	Part of inoculation	On normal surface			In punctures		
				With sclerotia	With mycelia	Control	With sclerotia	With mycelia	Control
I	Length of plants 40-74 cm Panicles began to come out	Sept. 9	1) Ligula	A 8	8	5	8	8	5
				B 5	6	0	3	6	0
				C 62.5	75.0	0.0	37.5	75.0	0.0
			2) Blade sheaths	A 10	9	5	10	9	5
				B 7	9	0	7	9	0
				C 70.0	100.0	0.0	70.0	100.0	0.0
II	Length of plants 52-74 cm Panicles began to come out	Sept. 11	3) Ligula	A 3	3	3	4	7	3
				B 2	2	0	3	7	0
				C 66.7	66.7	0.0	75.0	100.0	0.0
			4) Blade sheaths	A 8	5	5	4	6	5
				B 7	5	0	1	6	0
				C 87.5	100.0	0.0	25.0	100.0	0.0

1). 2 days after, water-soaked lesions with dark brown colour were formed. 2). 2 days after, water-soaked lesions with purplish brown colour were formed and within 2 weeks its center became cloudy or grayish brown and the margin dark brown. Using mycelia small light yellow sclerotia were formed. 3). do. 4). do.

TABLE 7 *The results of inoculation with sclerotia or mycelia on paddy rice*

A, No. of plants inoculated. B, No. of plants infected. C, % of plants infected.

No. of exp.	Plants	Date of inoculation	Part of inoculation	On normal surface			In punctures		
				With sclerotia	With mycelia	Control	With sclerotia	With mycelia	Control
I	Length of plants 60-85 cm	Sept. 9	1) Ligula	A 3 B 2 C 66.7	A 3 B 2 C 66.7	A 3 B 0 C 0.0	A 3 B 3 C 100.0	A 3 B 2 C 66.7	A 3 B 0 C 0.0
	Kernel containing milky juice			A 5 B 0 C 0.0	A 5 B 2 C 40.0	A 5 B 0 C 0.0	A 5 B 3 C 60.0	A 5 B 1 C 20.0	A 5 B 0 C 0.0
			2) Blade sheaths						
II	Length of plants 45-95 cm	Sept. 11	3) Ligula	A 3 B 2 C 66.7	A 3 B 3 C 100.0	A 3 B 0 C 0.0	A 3 B 3 C 100.0	A 3 B 3 C 100.0	A 3 B 0 C 0.0
	Kernel containing milky juice			A 5 B 2 C 40.0	A 4 B 2 C 50.0	A 5 B 0 C 0.0	A 5 B 2 C 40.0	A 5 B 3 C 60.0	A 5 B 0 C 0.0
			4) Blade sheaths						

1). 2 days after, water-soaked lesions with purplish brown colour were produced on affected areas and the leaves wilted.

4). Red or grayish brown cloudy lesions were produced on the affected areas and the leaves wilted.

TABLE 8

The results of inoculation with sclerotia or mycelia on various plants

A, No. of plants inoculated. B, No. of plants infected. C, % of plants infected.

Name of plants	Plants	Date of inoculation		On normal surface			In punctures			Remarks
				With sclerotia	With mycelia	Control	With sclerotia	With mycelia	Control	
Tomato	Length of plants 36-48 cm Flower open	June 10	A B C	6 6 100.0	6 6 100.0	6 0 0.0	6 6 100.0	6 6 100.0	6 0 0.0	2 days after, affected areas produced water-soaked lesions with brown colour, gum excreted, and within 2 weeks, epidermis was split there, the lesions were deeply sunken, and the whole plant began to wilt.
Pepper	Length of plants 9-24 cm Flower open	June 17	A B C	12 0 0.0	12 2 16.7	12 0 0.0	12 2 16.7	12 6 50.0	12 0 0.0	2 days after, affected areas formed reddish brown coloured lesion, within 2 weeks deeply sunken, and later the leaves dropped.
Egg-plant	Length of plants 7-12 cm	June 17	A B C	6 2 33.3	6 7 33.3	6 0 0.0	6 1 16.7	6 3 50.0	6 0 0.0	Affected areas reddish brown turning later to grayish brown, and became slightly sunken.
Broad beans	Length of plants 15-36 cm	June 17	A B C	8 1 12.5	8 1 12.5	8 0 0.0	8 6 75.0	8 4 50.0	8 0 0.0	2 weeks after, numerous white sclerotia were formed on affected areas.
Squash	Length of plants 6-8 cm	June 27	A B C	22 20 90.9	22 0 0.0	22 0 0.0	22 20 90.9	22 0 0.0	22 0 0.0	2 weeks after, affected leaves began to drop, and brown sclerotia were formed.
Cucumber	Length of plants 5-7 cm Number of leaves 3-4	June 27	A B C	6 6 100.0	6 4 66.7	6 0 0.0	6 6 100.0	6 4 66.7	6 0 0.0	2 days after, affected areas changed to irregular shaped lesion light red in colour. Petiole began to be broken and within 2 weeks, all affected plants died completely, and brown sclerotia were formed.
"Sennarihyôtan" <i>Lagenaria vulgaris</i> var. <i>microcarpa</i>	Length of plants 7-10 cm Number of leaves 3-4	June 29	A B C	7 4 57.1	7 5 71.4	7 0 0.0	7 5 71.4	7 5 71.4	7 0 0.0	2 days after, affected areas changed to brown, water-soaked lesion, gum excreted, and within 2 weeks numerous sclerotia were formed.
"Yûgao" <i>Lagenaria vulgaris</i> var. <i>clavata</i>	Length of plants 9-12 cm Number of Leaves 3-4	June 29	A B C	8 6 75.0	8 2 25.0	6 0 0.0	8 3 37.5	7 2 28.6	6 0 0.0	Surface of affected areas split and began to wilt.
Water melon	Length of plants 8-10 cm Number of leaves 3-4	July 1	A B C	4 4 100.0	4 4 100.0	3 0 0.0	4 4 100.0	4 1 25.0	3 0 0.0	Surface of affected areas became a water-soaked lesion, gum excreted, and the stem was broken at the lesion. Within 2 weeks, all affected plants died completely, and sclerotia were formed.
"Hechima" <i>Luffa cylindrica</i>	Length of plants 7-10 cm Number of leaves 2-3	July 1	A B C	4 0 0.0	4 0 0.0	3 0 0.0	4 4 100.0	4 0 0.0	3 0 0.0	do.
Sweet potato	Length of plants 7-25 cm Number of leaves 3-10	Aug. 1	A B C	20 7 35.0	20 17 85.0	20 0 0.0	20 3 15.0	20 17 85.0	20 0 0.0	2 weeks after, affected areas turned to grayish brown, epidermis split.

As Table 8 is the result of only one experiment, further studies are to be made in order to secure the more precise conclusions. It seems that tomato, cucumber, water melon and "sennarihyôtan" (*Lagenaria vulgaris* var. *microcarpa*) are more susceptible to the disease than sweet potato, squash, "yôgao" (*Lagenaria vulgaris* var. *clavata*), broad beans, egg plant, "hechima" (*Luffa cylindrica*) and pepper.

Summary

1. In this paper the results of study on the morphology, physiology and pathogenicity of *Corticium centrifugum* (Lév.) Bres. causing Shirakinu disease of calabash (*Lagenaria vulgaris* var. *depressa*) are reported.

2. The causal fungus attacks the stem of calabash in the field in about the middle of August, and on affected areas first a mycelial mass is found. As the disease progresses, the affected areas undergo conspicuous change in colour, become deeply sunken, and later numerous sclerotia are formed there. The affected areas and often the stem portion above them die or wilt.

3. Using twenty different media, it was found that very vigorous growth of the mycelia and the abundant formation of sclerotia occurred on agar and nutrient of soy and apricot. Asparagin synthetic and Richard's solution were very poor in these respects, and a characteristic mycelial layer was produced on agar medium of the former. On soy agar after Miyoshi's formula large irregular shaped and flattened sclerotia were produced, and in the same nutrient media mycelium did not grow at all.

4. The minimum temperature for the mycelial growth on apricot decoction agar, soy agar and carrot decoction agar lies between 11° and 15°C, the optimum is about 28°C, and the maximum seems to be above 38°C.

5. This fungus attacks also the rice plant (paddy and upland), tomato, pepper, broad beans, squash, cucumber, "sennarihyôtan" (*Lagenaria vulgaris* var. *microcarpa*), "yôgao" (*Lagenaria vulgaris* var. *clavata*), water melon, "hechima" (*Luffa cylindrica*) and sweet potato.

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Explanation of Plate I

- Fig. 1 *Corticium centrifugum* on calabash stem.
- Fig. 2 *Corticium centrifugum* on calabash decoction agar.
- Fig. 3 Inoculation experiment on the stem of water melon seedlings with *Corticium centrifugum*. a. Healthy seedlings as control. b. Affected seedlings.

Fig. 1

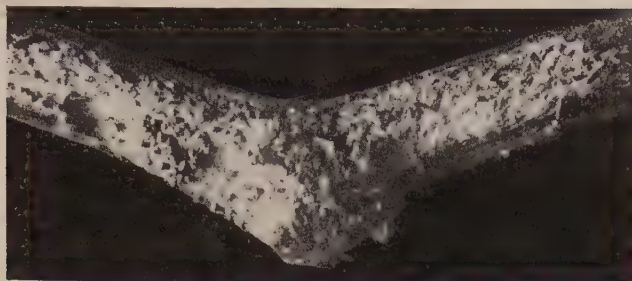


Fig. 2

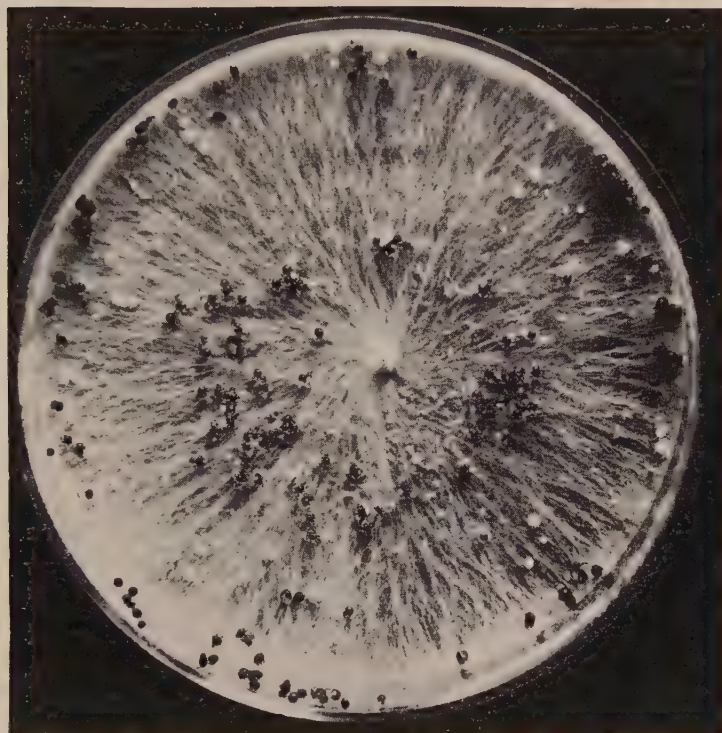


Fig. 3



Vitality of *Corticium centrifugum* Parasitic on Calabash Plant

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Introduction

In the preceding paper (4), the writer reported the results of studies on the morphology, physiology as well as pathogenicity of *Corticium centrifugum* (Lév.) Bres. causing Shirakinu disease of Calabash (*Lagenaria vulgaris* var. *depressa*).

In the present paper, the vitality of the same fungus with special reference to the overwintering and the resistance to high temperature and various chemicals is dealt with.

The writer wishes to thank to Mr. S. Higeta for his assistance throughout the whole course of this study.

Overwintering of the Causal Fungus

For the study of overwintering of the causal fungus, the following experiments were carried out.

A. In the soil

The unglazed pots (18 cm in dia.) filled with soil were sterilized in Koch's steam sterilizer three times on alternate days, each for a period of 2 hours. 20 mature sclerotia and 4 pieces of mycelial layer wrapped up together with a sterilized cloth were put in each of these pots, at the depth of 3 and 9 cm from the surface of the soil.

These pots were buried on Nov. 14, 1930 in the sunny field, so as to place the surface of the soil in pots at the level of the ground.

With every month's interval thereafter, the sclerotia and mycelia were taken out of the pots, washed thoroughly with sterilized distilled water and transferred immediately to apricot decoction agar in Petri dishes, and incubated at about 24°C for 10 days. Their vitality was decided by studying the growth of mycelia and the staining reaction of sclerotia with 1 % eosin solution.

The results are given in Table 1 in which the numbers show those of sclerotia and mycelia survived, — presents that all materials were found dead and * indicates that the strict decision of vitality could not be made owing to various causes in the experiment.

TABLE 1 *The vitality of the causal fungus in soil*

Depth (cm)	3		6	
Fungi	Sclerotia	Mycelia	Sclerotia	Mycelia
Time				
Dec. 1930	20	4	20	4
Jan. 1931	20	4	20	4
Feb. "	17	4	20	4
Mar. "	20	4	20	4
Apr. "	19	*	20	*
May "	20	4	20	*
June "	20	4	20	4
July "	*	*	*	*
Aug. "	*	*	2	*
Sep. "	*	2	2	*
Oct. "	*	*	15	*
Nov. "	*	*	1	*

As above Table shows, the causal fungus in the soil has undergone no special change to June of the following year. Afterwards the fungus seems to decrease its vitality more or less rapidly, though the germinating power of a sclerotium was kept intact for one year.

Endo (1,2) and Yokogi (3) reported on the overwintering in the soil

of the sclerotia and mycelia of the same fungus obtained from different kinds of hosts. The results of the writer agree in main with those of their studies.

B. On host plants

On Sept. 3, 1930, the affected stems of calabash were collected and they were kept in the laboratory in air dry state. On Sep. 3, Nov. 9, 1931 and Jan. 23, 1932, their vitality was carefully examined in the same manner as in A, and it was found that all sclerotia had survived very well.

C. On culture media

On Sept. 12, 1930, the writer cultured this fungus on calabash decoction agar in Erlenmyer flasks and kept them in the laboratory. On Sep. 12, Nov. 9, 1931 and Jan. 23, 1932, careful examinations were made by the same method as described before, and all sclerotia were found to have survived completely.

Effect of High Temperature upon the Vitality of the Causal Fungus

The study of thermal death point of pathogenes is of importance from both the practical and scientific points of view. At first the causal fungus was grown on apricot decoction agar in Erlenmyer flasks for 25 days at about 24°C.

Air bubbles adhering to the sclerotia were removed from them by shaking Petri dishes containing sclerotia and sterilized distilled water. These sclerotia and a piece of mycelial layer were put into the test tubes containing 5 cc of sterilized distilled water and also into those without water.

The tubes were exposed to different temperatures in a water bath and dry-sterilizer during different length of time. After this treatment, the sclerotia and mycelia were taken out by a platinum needle, and transferred immediately to apricot decoction agar in Petri dishes and incubated at about 24°C for 10 days.

The thermal death point of the fungus was decided by the same method as described before. The results are given in Tables 2-5. In the experiments shown in Tables 2, 3 and 4, 10 sclerotia or 4 pieces of mycelial layer were put in each test tube, while in the experiment presented in Table 5, 2 pieces of mycelial layer were used. The numbers in each Table show those of sclerotia and mycelial pieces found alive, and - indicates that the materials were dead.

TABLE 2 *Effect of high temperature upon the vitality of sclerotia under water*

Temperature (C)	No. of exp.	Control	Time (m)								
			10	20	30	40	50	60	70	80	90
45	I	10	10	10	10	10	10	10	10	10	10
	II	10	10	10	10	10	10	10	10	10	10
	III	10	10	10	10	10	10	10	10	10	10
50	I	10	8	6	4	3	—	—	—	—	—
	II	10	9	5	—	—	—	—	—	—	—
	III	10	10	9	3	—	—	—	—	—	—
55	I	10	—	—	—	—	—	—	—	—	—

TABLE 3 *Effect of high temperature upon the vitality of mycelia under water*

Temperature (C)	No. of exp.	Control	Time (m)								
			10	20	30	40	50	60	70	80	90
45	I	4	4	1	—	—	—	—	—	—	—
	II	4	4	1	—	—	—	—	—	—	—
50	I	4	—	—	—	—	—	—	—	—	—
	II	4	—	—	—	—	—	—	—	—	—

TABLE 4 *Effect of high temperature upon the vitality of sclerotia in the air*

Temperature (C)	No. of exp.	Control	Time (m)								
			10	20	30	40	50	60	70	80	90
60	I	10	10	7	2	—	2	—	—	—	—
	II	10	10	5	—	—	1	—	—	—	—
70	I	10	—	2	—	—	—	—	—	—	—
	II	10	—	—	1	—	—	—	—	—	—
80	I	10	—	—	1	—	—	—	—	—	—
	II	10	—	—	—	—	—	—	—	—	—

TABLE 5 *Effect of high temperature upon the vitality of mycelia in the air*

Temperature (C)	No. of exp.	Control	Time (m)								
			10	20	30	40	50	60	70	80	90
60	I	2	2	—	—	—	—	—	—	—	—
	II	2	1	—	—	—	—	—	—	—	—

70	I II	2 2	—	—	—	—	—	—	—	—	—
80	I II	2 2	—	—	—	—	—	—	—	—	—

Thus, in the water, the sclerotia were killed in 50 minutes at 50°C, and the mycelia in 30 minutes at 45°C. In the air on the other hand, the sclerotia were killed in 60 minutes at 60°C, 40 minutes at 70° and 80°C, and the mycelia in 20 minutes at 60°C.

Effect of Various Chemicals upon the Causal Fungus

Some experiments were carried out in order to determine the effect of various chemicals upon the causal fungus.

The test tubes were previously immersed in a solution composed of potassium bichromate 60 gr, sulphuric acid 60 gr, distilled water 1000 cc, and then washed thoroughly with tap water and then with sterilized distilled water. 5-10 sclerotia and 2-3 pieces of mycelial layer, which were grown on apricot decoction in Erlenmyer flasks kept at about 24°C for 30 days, were put into test tubes containing 2 cc of chemicals which are described below. Controls were made using the same volume of distilled water.

The chemicals used in the study are as follows:-

Copper sulphate, corrosive sublimate, formalin, milk of lime, caustic potash, alcohol. All of these were used as water solutions of different concentrations.

Flowers of sulphur. This was used as powder.

Ashes A. The straw and charcoal ash water solution.

B. The straw and charcoal ash decoction.

C. The pine-needle ash decoction.

Bordeaux mixtures

A. Copper sulphate 0.83 gr, quick lime 0.83 gr, dist. water 100 cc.

B. Copper sulphate 0.62 gr, quick lime 1.24 gr, dist. water 100 cc.

C. Copper sulphate 0.83 gr, quick lime 0.42 gr, dist. water 100 cc.

Self-boiled lime sulphur: Flowers of sulphur 25 gr, quick lime 25 gr, dist. water 100 cc. Original solution was 5° (Baume).

After different length of time the sclerotia and mycelia were taken out with a platinum needle, washed carefully with sterilized distilled water

and transferred to agar media. For the materials treated with corrosive sublimate, copper sulphate, formalin, ashes, Bordeaux mixtures and self-boiled lime sulphur, the apricot decoction agar was used, and for those treated with milk of lime, alcohol, flowers of sulphur, caustic potash, the carrot decoction agar was employed. The control materials from distilled water were grown on the corresponding medium on which the treated materials were grown.

They were all incubated at about 24°C for 10 days and the vitality was determined. The results are shown in Tables 6-10, in which the numbers present those of the sclerotia and mycelial pieces found alive, + indicate that all materials treated survived, while - means that they were all dead. In controls all the materials were kept intact, so that the numbers are equal to those of the materials used.

TABLE 6 *Effect of copper sulphate upon the vitality of sclerotia and mycelia*

Chemicals	Fungi	% of solution	No. of exp.	Room temp. (C)	Control	Time (m)			
						30	60	120	240
Copper sulphate	Sclerotia	2	I	In exp. I 16°-20°	10	+	+	+	+
			II		10	+	+	+	+
		5	I		10	+	+	+	+
			II		10	+	+	+	+
		10	I		10	+	+	+	+
			II		10	+	+	+	+
		15	I	In exp. II 16°-18°	10	+	+	+	+
			II		10	+	+	+	+
		20	I		10	+	+	+	+
			II		10	+	+	+	+
	Mycelia	2	I	In exp. I 16°-20°	2	+	+	+	+
			II		2	+	+	+	+
		5	I		2	+	+	+	+
			II		2	+	+	+	1
		10	I	In exp. II 16°-18°	2	+	+	+	-
			II		2	+	+	+	-
		15	I		2	+	1	-	-
			II		2	+	1	-	-
		20	I		2	+	1	-	-
			II		2	+	1	-	-

TABLE 7 *Effect of corrosive sublimate, formalin and milk of lime upon the vitality of sclerotia and mycelia*

Chemicals	Fungi	% of solution	No. of exp.	Room temp. (C)	Control	Time (m)							
						15	30	45	60	90	120	150	180
Corrosive sublimate	Sclerotia	0.01	I	In exp. I 16°	7	+	+	+	+	+	+	+	+
			II		7	+	+	+	+	+	+	+	+
		0.05	I	In exp. II 14°	7	+	+	+	+	+	2	2	2
			II		7	+	+	+	+	+	3	2	2
		0.1	I		7	+	+	-	-	-	-	-	-
			II		7	+	+	-	-	-	-	-	-
	Mycelia	0.01	I	In exp. I 16°	3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
		0.05	I	In exp. II 14°	3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
		0.1	I		3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
Formalin	Sclerotia	0.5	I	In exp. I 17°-19°	7	+	+	+	+	+	2	-	-
			II		7	+	+	+	+	6	3	-	-
		1.0	I	In exp. II 21°-22°	7	+	+	+	-	-	-	-	-
			II		7	+	+	+	-	-	-	-	-
		3.0	I		7	+	-	-	-	-	-	-	-
			II		7	6	-	-	-	-	-	-	-
	Mycelia	0.5	I	In exp. I 17°-20°	3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
		1.0	I	In exp. II 18°-22°	3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
		3.0	I		3	+	-	-	-	-	-	-	-
			II		3	+	-	-	-	-	-	-	-
Milk of lime	Sclerotia	5	I	In exp. I 24°-25°	5	+	+	+	+	+	-	-	-
			II		5	+	+	+	+	+	-	-	-
		10	I	In exp. II 23°-26°	5	+	+	+	+	2	-	-	-
			II		5	+	+	+	+	1	-	-	-
		20%	I		5	+	+	+	+	+	-	-	-
			II		5	+	+	+	+	3	-	-	-
	Mycelia	5	I	In exp. I 25°-26°	3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
		10	I	In exp. II 24°-26°	3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
		20	I		3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-

TABLE 8 *Effect of caustic potash, alcohol and flowers of sulphur upon the vitality of sclerotia and mycelia*

Chemicals	Fungi	% of solution	No. of exp.	Room temp. (C)	Control	Time (m)							
						15	30	45	60	90	120	150	180
Caustic potash	Sclerotia	0.5	I	In exp. I 16°-17°	5	+	+	+	+	+	+	+	+
			II		5	+	+	+	+	+	+	+	+
		1.0	I		5	-	-	-	-	-	-	-	-
			II	In exp. II 16°-18°	5	-	-	-	-	-	-	-	-
		2.0	I		5	-	-	-	-	-	-	-	-
			II		5	-	-	-	-	-	-	-	-
	Mycelia	0.5	I	In exp. I 16°-17°	3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
		1.0	I		3	-	-	-	-	-	-	-	-
			II	In exp. II 17°-18°	3	-	-	-	-	-	-	-	-
		2.0	I		3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
Alcohol	Sclerotia	20	I	In exp. I 16°-19°	5	+	+	+	+	+	+	+	+
			II		5	+	+	+	+	+	+	+	+
		30	I		5	+	+	+	+	+	-	-	-
			II	In exp. II 15°-17°	5	+	+	+	+	+	-	-	-
		40	I		5	+	-	-	-	-	-	-	-
			II		5	+	-	-	-	-	-	-	-
	Mycelia	20	I	In exp. I 16°-20°	3	+	+	-	-	-	-	-	-
			II		3	+	+	-	-	-	-	-	-
		30	I		3	-	-	-	-	-	-	-	-
			II	In exp. II 16°-19°	3	-	-	-	-	-	-	-	-
		40	I		3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
Flowers of sulphur	Sclerotia		I	In exp. I 20°-26°	5	+	+	+	+	+	+	+	+
			II	In exp. II 21°-27°	5	+	+	+	+	+	+	+	+
	Mycelia		I	In exp. I 21°-28°	3	-	-	-	-	-	-	-	-
			II	In exp. II 20°-27°	3	-	-	-	-	-	-	-	-

TABLE 9 *Effect of ashes upon the vitality of sclerotia and mycelia*

Chemicals	Fungi	% of solution	No. of exp.	Room temp. (C)	Control	Time (m)							
						15	30	45	60	90	120	150	180
Ash (A)	Sclerotia	20	I	In exp. I 21°-22°	5	+	+	-	-	-	-	-	-
			II		5	+	+	-	-	-	-	-	-
			I		5	+	+	-	-	-	-	-	-
		30	II	In exp. II 25°-26°	5	+	3	-	-	-	-	-	-
			I		5	+	+	-	-	-	-	-	-
			II		5	+	+	-	-	-	-	-	-
	Mycelia	20	I	In exp. I 21°-23°	3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
			I		3	-	-	-	-	-	-	-	-
		30	II	In exp. II 24°-25°	3	-	-	-	-	-	-	-	-
			I		3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
Ash (B)	Sclerotia	20	I	In exp. I 25°-27°	5	2	-	-	-	-	-	-	-
			II		5	-	-	-	-	-	-	-	-
			I		5	-	-	-	-	-	-	-	-
		30	II	In exp. II 27°-28°	5	-	-	-	-	-	-	-	-
			I		5	2	-	-	-	-	-	-	-
			II		5	3	-	-	-	-	-	-	-
	Mycelia	20	I	In exp. I 26°-27°	3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
			I		3	-	-	-	-	-	-	-	-
		30	II	In exp. II 26°-27°	3	-	-	-	-	-	-	-	-
			I		3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
Ash (C)	Sclerotia	10	I	In exp. I 27°	5	+	+	+	+	+	+	+	+
			II		5	+	+	+	+	+	+	+	+
			I		5	+	+	+	+	+	+	+	+
		20	II	In exp. II 27°	5	+	+	+	+	+	+	+	+
			I		5	+	+	+	+	+	+	+	+
			II		5	+	+	+	+	+	+	+	+
	Mycelia	10	I	In exp. I 22°	3	+	+	+	+	+	+	+	+
			II		3	+	+	+	+	+	+	+	+
			I		3	+	+	+	+	+	+	+	+
		20	II	In exp. II 28°	3	+	+	+	+	+	+	+	+
			I		3	+	+	+	+	+	+	+	+
			II		3	+	+	+	+	+	+	+	+

TABLE 10 *Effect of Bordeaux mixture and self-boiled lime sulphur upon the vitality of sclerotia and mycelia*

Chemicals	Fungi	% of solution	No. of exp.	Room temp. (C)	Control	Time (m)							
						15	30	45	60	90	120	150	180
Bordeaux mixture	Sclerotia	A	I	In exp. I 17°-20°	5	+	+	+	+	+	1	1	-
			II		5	+	+	+	+	+	-	-	-
			I		5	+	+	+	+	-	-	-	-
			II		5	+	+	+	+	-	-	-	-
		C	I	In exp. II 18°-20°	5	+	+	+	+	-	-	-	-
			II		5	+	+	+	+	-	-	-	-
			I		5	+	+	+	+	-	-	-	-
			II		5	+	+	+	+	-	-	-	-
	Mycelia	A	I	In exp. I 16°-18°	3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
			I		3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
		B	I	In exp. II 17°-20°	3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
			I		3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
Self-boiled lime sulphur	Sclerotia	0.5	I	In exp. I 13°-15°	5	+	+	+	+	+	+	+	+
			II		5	+	+	+	+	+	+	+	+
			I		5	+	+	+	+	+	+	+	+
			II		5	+	+	+	+	+	+	+	+
		1.0	I	In exp. II 12°-15°	5	+	+	+	+	+	+	+	+
			II		5	+	+	+	+	+	+	+	+
			I		5	+	+	+	+	+	+	+	+
			II		5	+	+	+	+	+	+	+	+
	Mycelia	0.5	I	In exp. I 14°-17°	3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
			I		3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
		1.0	I	In exp. II 13°-15°	3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-
			I		3	-	-	-	-	-	-	-	-
			II		3	-	-	-	-	-	-	-	-

In general, the order of injury of chemicals for the causal fungus may be said to be as follows; corrosive sublimate, formalin, caustic potash, ashes (A, B), milk of lime, Bordeaux mixture, self-boiled lime sulphur, alcohol, flowers of sulphur, copper sulphate and ash (C). The mycelia were more sensitive to chemicals than sclerotia. Ashes (A) and (B) were more injurious than ash (C). This result seems to be an interesting problem for the further study.

Summary

1. In this paper, the results of study on the overwintering as well as the resistance to high temperature and various kinds of chemicals of *Corticium centrifugum* (Lév.) Bres. causing Shirakinu disease of calabash (*Lagenaria vulgaris* var. *depressa*) are reported.

2. The sclerotia and mycelia in the soil, on the host plant and on the culture media passed the winter alive at Utsunomiya.

3. Under the water, the sclerotia were completely killed in 50 minutes at 50°C, and mycelia in 30 minutes at 45°C. But in the air, the sclerotia were killed in 60 minutes at 60°C, and mycelia in 20 minutes at 60°C.

4. The order of injury of chemicals for the sclerotia and mycelia may be as follows; corrosive sublimate, formalin, caustic potash, ashes (A, B), milk of lime, Bordeaux mixture, self-boiled lime sulphur, alcohol, flowers of sulphur, copper sulphate and ash (C).

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單色光線の酵母の醗酵生成物に及ぼす影響

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The Effect of Monochromatic Lights on the Fermentation
Products of the Yeasts

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緒 言

紫外線の影響に關しては幾多の研究ありと誰も、可視光線に關する研究は極めて少なく、E. Purvis and R. Warwick (1) (2) は $\alpha = 16^\circ$ の葡萄糖を含める hopped wort に *Saccharomyces cerevisiae* を接種し、 $21.3^\circ - 22.3^\circ\text{C}$ の定溫器内に保ち、醗酵終了後 N_{10} alkali を用ひて其酸度を檢したるに dark 12.3 cc, white 12.5 cc, red 12.3 cc, blue 23.8 cc なりしといふ。Lubimenco et Troloff-Bagreief (3) は糖 24.68% を含む葡萄汁を暗黒に於て醗酵せしむれば、糖の 24.3% を消失して 14.4 vol.% の alcohol を生ずるも、光線下に於ては 20.9% の糖消失して 12.11 vol. % の alcohol を生じ、又發生する CO_2 も光線下に於ては暗所に於けるよりも僅に少なく、葡萄酒を分析せしに酸の量特に揮發酸の量は光線下に於けるもの暗所の夫れよりも高く、glycerin は其反對にして、實驗の終に於ける酵母の乾物量及酒中の esters 量は兩者同量なりしと報じ、以て alcohol の生成は暗所に於て有利に、酸特に揮發酸の生成は光線下に於て多しとせり。

著者は麴浸出液に dried yeasts を接種し、單色光線の影響下に於ける醗酵生成物に就て研究したれば、茲に其一端を發表し識者の叱正を乞はんとす。

實 驗

〔I〕 装置, 培養基及菌種

1. 單色光線濾膜：赤色, 黃色, 綠色 I 及 II, 青色 I 及 II, 紫色 I 及 II, 比較の爲めの白色及黑色, 合せて 10 種の濾膜を用ふ。白色光線濾膜は gelatine 溶液のみにして, 赤色より黑色に至る 9 種の濾膜は

Red	Acid rhodamine	1.0	Yellow	Tartrazine	1.0
	Erythrosine	1.0			
	Tartrazine	1.0			
Green I	Patent blue	0.3	Green II	Patent blue	1.0
	Tartrazine	1.0		Tartrazine	1.0
Blue I	Patent green	1.0	Blue II	Acid rhodamine	0.3
	Toluidine blue	0.47		Patent green	1.0
	Filter yellow	0.33			
Violet I	Crystal violet	1.0	Violet II	Methyl violet	1.0
	Toluidine blue	0.2		Toluidine blue	0.4
Black		India ink	1.0		

の割合にて調合したる色素をば 10% gelation 溶液に溶かし, 2 枚の硝子板に挟みたるものにして, 各濾膜の透過波長は次の如し(Plate II 参照)。

名 稱	透過波長 (I. Å.)	透過光線の色
白	全可視光線	白
赤	6130 以上	赤
黄	6700-(6130-5600)-4900	赤-(帶赤黄-帶綠黄)-帶青綠
綠 I	5660-5040	帶黄綠-綠
綠 II	5460-4890	帶黄綠-帶青綠
青 I	5050-4730	綠-帶紫青
青 II	4960-4530	帶綠青-帶紫青
紫 I	4690-3820	帶青紫-紫
紫 II	4410-3960	紫
黑	無 し	暗黑

此等の光線濾膜は赤外線透過を許すも紫外線透過を許さざることは、分光寫眞によりて豫め確め置きたり。(尙ほ單色光線濾膜に就ては他の機會に於て之が發表を期す)。

2. 光線濾過槽：7×10×20 cm なる長方形の金屬槽にして内外面は黒塗とし、面前には上述の單色光線濾膜を挿込み、隙間より他の光線の侵入せざるやう黒羅紗及黒綿の押へを施し、上面には空氣の流通を許す爲め24の圓孔を穿ち、其内面には黒羅紗二枚及黒綿の厚層をあて以て光線の侵入を防ぎたれば、槽内には濾膜を通過したる光線のみ射入す。

3. 光源：100燭光の瓦斯入 Tungsten 電球を用ひ、光源と濾膜との距離を175 cm とす。

4. 培養基：麴浸出液 5 cc 宛を試験管にとり、常法の如く滅菌したる液體培養基にして、100 cc 中

Sugar (as glucose) (g)	Crude protein (g)	Ash (g)	Acidity (cc of N/10 NaOH wanted for neutral.)
9.820	0.432	0.051	13.50

を含み、又之を蒸餾操作に付して、殘液及餾出液を検するに、原液 100 cc に對し

Original acidity	Residual liquid	Distillate				
	Acidity	Acidity	Alcohol	Aldehyde (mg)	Acetal	Ester (mg)
13.50	14.50	0.32	0	0.352	0	2.112

なる結果を得、不揮發酸の増加量 $N/10$ NaOH の 1 cc に相當し、餾出液には揮發酸の 0.32 cc $N/10$ NaOH 相當量、aldehyde の 0.352 mg 及 ester の 2.112 mg を含むも、alcohol 及 acetal は含有せざるを知れり。後記實驗成績に掲載の揮發酸、aldehyde、ester の量は此等の含有量を夫々扣除したる差を示せるものなり。

更に本實驗期間中單色光線によりて培養基の組成分に變化を及ぼすや否やを検する爲め、本實驗と同一狀態の下に培養基のみを單色光線に曝露し、48時及96時の後分析に付したれども、殘液及餾出液の酸度は前表と一致し、aldehyde 及 ester も實驗誤差の範圍内にして、之亦變化を認め難く、alcohol 及 acetal の生成を見ず。又培養基の pH

(=5.1)にも實驗誤差の範圍を考ふれば變化を認めざりしが故に、著者の實驗に於けるが如き短期間にありては、單色光線により培養基の組成分に變化を及ぼせりと思推するを得ず。

5. 供試酵母 : *Saccharomyces cerevisiae* 及 *S. ellipsoideus* の2種にして、何れも純系を分離し、Pasteur's flask にて純粹培養をなしたるものにつき、濾過、洗滌後壓搾により表面の附著水分を除きたる後、無水酒精及 ether 混合液(3:1)にて洗ひ、最後に ether にて洗ひたるものをば、電氣定溫器内にて24時間45°Cにて乾かしたるものを使用す。

6. 定溫器 : 其溫度を約25°Cに保ち、接種培養基を入れたる光線濾過槽を靜置し、光源よりの光線を十分に槽の前面に照射せしむ。

〔II〕 酵母の培養及其培養基の分析

各培養管に對し dried yeasts 0.0025 g宛を接種したるものを上述の裝置の如くして定溫器内に保ち、單色光線下に醗酵せしめたる後、48及96時を經過する毎に各槽より培養基を取出し、生成せられたる alcohol, aldehyde, acetal, ester, 揮發酸, 不揮發酸及總酸の定量をなす。其法は常法によりて蒸餾したる後、前五者は其餾出液より、不揮發酸は蒸餾殘液より其量を求むること次の如し:—

1. Alcohol : pycnometer を用ひ、比重法によりて其量を定め、100 cc 中に於ける g にて表はす。

2. Aldehyde : $N_{/200}$ K-metabisulphite を15分間反應せしめたる後、 $N_{/200}$ iodine にて滴定し、其差により aldehyde をば原液 100 cc 中に於ける mg にて表はす。

3. Acetal : $N_{/5}$ H_2SO_4 にて30分間分解反應を行はしめたる後、 $N_{/5}$ NaOH にて精密に中和し、 $N_{/200}$ K-metabisulphite を加へ15分間を経て、前法の如く 沃素液にて滴定し、aldehyde の場合に於ける滴定數との差を以て acetal となし、原液 100 cc 中に於ける mg にて表はす。

4. Ester : $N_{/10}$ NaOH を加へ24時間放置、鹼化せしめたる後同容の $N_{/10}$ H_2SO_4 を加へ、 $N_{/20}$ NaOH にて中和し、其要したる cc 數より揮發酸中和量を減じ、其差を ethyl ester として計算し、100 cc 中に於ける mg にて表はす。

5. 揮發酸 : $N_{/20}$ NaOH にて滴定し、原液 100 cc 中に於ける醋酸の mg として表はす。

6. 不揮發酸 : 中和に要する $N_{/20}$ NaOH の cc 數を定め、蒸餾操作によりて増加する酸量及原液中に含みし酸量を減じ、其差を以て原液 100 cc 中に於ける琥珀酸の mg と

して表はす。

7. 總 酸：揮發酸及不揮發酸の和をば $N_{/10}$ NaOH の cc 數にて表はす。

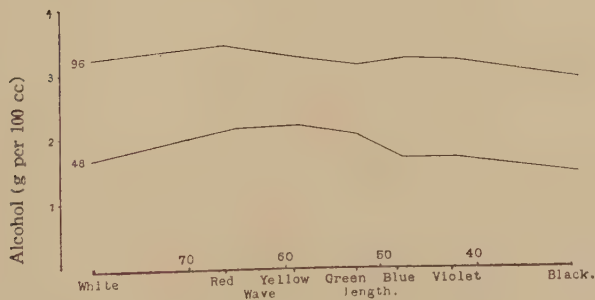
〔III〕 實驗成績

各色光線下に於て生成せらるる醗酵産物の含有量は次の如し：—

1. Alcohol (g per 100 cc)

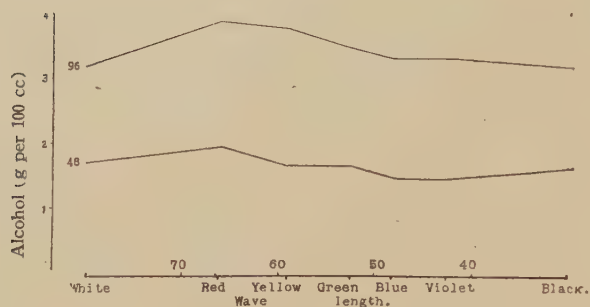
Name of yeast Lapsed time Name of filter	<i>S. cerevisiae</i>		<i>S. ellipsoideus</i>	
	48	96	48	96
White	1.651	3.200	1.699	3.174
Red	2.110	3.413	1.953	3.871
Yellow	2.151	3.220	1.683	3.779
Green {	I	3.428	1.477	3.423
	II	2.748	1.887	3.555
	Average	3.088	1.682	3.489
Blue {	I	3.066	1.567	3.359
	II	3.301	1.398	3.262
	Average	3.184	1.483	3.312
Violet {	I	3.158	1.306	3.385
	II	3.148	1.649	3.239
	Average	3.153	1.478	3.312
Black	1.405	2.874	1.652	3.166

Fig. 1. *S. cerevisiae*



之に依れば初めより alcohol の生成に有利なる光線は波長 6130 I. Å. 以上の赤色光線にして, 6130—5600 I. Å. の黄色光線, 5600—4890 I. Å. の緑色光

線之に次ぎ, 其生成に最不利なるは暗黒にして, 紫色及青色光線之に次で劣れるを見れば, alcohol の生成は 4900—6130 up I. Å. の如き長波長の光線下に於て多く, 短波

Fig. 2. *S. ellipsoideus*

長の光線下に於て少なきを知るべく又暗黒に於ては白色光線下に於けるよりも alcohol の生成少なく, 此點 Lubimenco et Troloff-Bagreief の成績と一致せず(Fig.1, Fig.2)。

2. Aldehyde (mg per 100 cc)

Name of yeast Lapsed time Name of filter	<i>S. cerevisiae</i>		<i>S. ellipsoideus</i>	
	48	96	48	96
White	1.452	2.728	0.836	6.688
Red	0.526	1.796	1.012	7.524
Yellow	0.484	2.178	0.264	4.268
Green	I	0.528	2.327	0.913
	II	0.616	1.496	0.880
	Average	0.572	1.912	0.897
Blue	I	1.408	3.344	1.095
	II	0.616	0.440	0.704
	Average	1.012	1.892	0.900
Violet	I	1.488	2.323	0.880
	II	0.190	2.002	1.056
	Average	0.839	2.163	0.968
Black	0.889	2.420	0.792	7.040

Fig. 3. *S. cerevisiae*

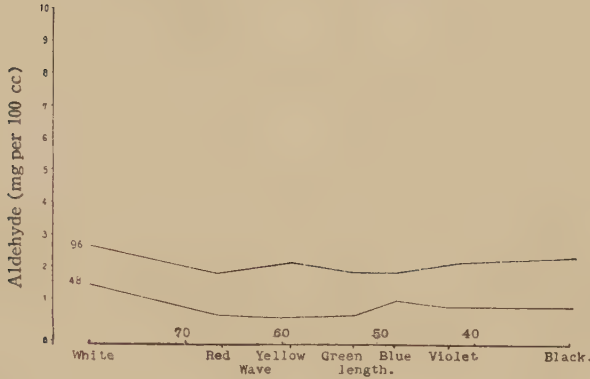
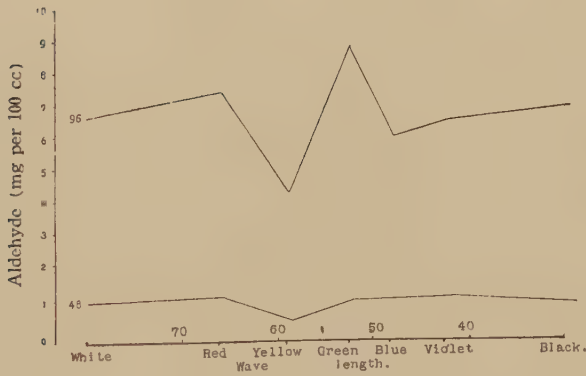


Fig. 4. *S. ellipsoideus*



之によれば, aldehyde の生成 *S. ellipsoideus* にありては, 常に白色光線下に於て多く初めは青色, 暗黒, 紫色の光線之に次げるも, 後には暗黒, 黄色, 紫色, 緑色, 青色の光線順次相次ぐを見, *S. ellipsoideus* にありては初め赤色及紫色光線下に於て優勢を示し, 青色及緑色光線之に次ぐも, 後には緑色光線下に於て最優位を示し, 赤色, 暗黒, 白色, 紫色, 青色等漸次之に次ぐに至る。されど黄色光線下に於ては初より終まで常に劣勢を現はせり。

以上三者を通じて aldehyde の生成は概して黄色光線下に於て少なく, 白色光線下に於て多し。又波長の傾

向よりすれば 4000—5600 I. Å. なる紫色乃至緑色光線下に於て, 其生成概して多きを示せり (Fig. 3, Fig. 4)。

3. Acetal (mg per 100 cc)

Name of yeast		<i>S. cerevisiae</i>		<i>S. ellipsoideus</i>	
Name of filter	Lapsed time	48	96	48	96
White		0	0.708	0	0
Red		0	0	0	0
Yellow		0	0.413	0	0.354
Green	I	0	0.236	0	0
	II	0	0	0	0
	Average	0	0.118	0	0
Blue	I	0	0	0	0
	II	0	1.434	0	0
	Average	0	0.717	0	0
Violet	I	0	0.354	0	0
	II	0	0.698	0	0
	Average	0	0.526	0	0
Black		0	0	0	0

之に依れば, dried yeasts は acetal を生成せざるか, 或は生成するも其量少なく且
不規則なり。白色, 黄色及紫色等の光線下に於て生成せられ易きにあらざるか。

4. Volatile acid (as acetic acid) (mg per 100 cc)

Name of yeast		<i>S. cerevisiae</i>		<i>S. ellipsoideus</i>	
Name of filter	Lapsed time	48	96	48	96
White		2.514	8.356	3.334	8.411
Red		4.685	9.344	3.943	9.715
Yellow		3.451	10.617	3.334	7.759
I		6.199	10.166	3.334	7.778

Green	II	3.794	8.170	3.334	9.688
	Average	4.997	9.168	3.334	8.733
Blue	I	6.199	11.228	4.060	10.351
	II	2.573	8.170	3.584	8.438
Violet	Average	4.388	9.699	3.822	9.395
	I	4.388	9.749	3.334	7.786
Black	II	4.388	9.449	3.885	8.438
	Average	4.388	9.599	3.610	8.112
Black		4.060	9.704	3.885	9.699

Fig. 5. *S. cerevisiae*

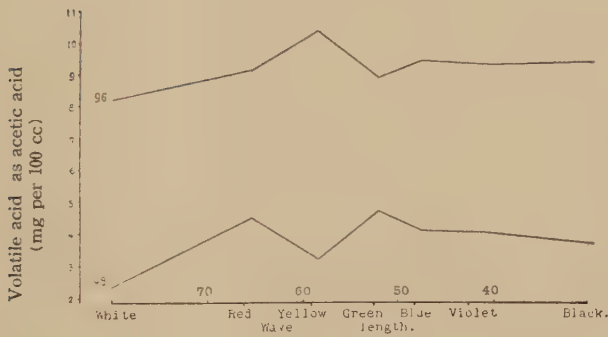


Fig. 6. *S. ellipsoideus*



之に依れば、揮發酸の生成は *S. cerevisiae* にありては、初め綠色光線下に於て多く、赤色、青色、紫色、暗黒之に次ぎ、後には黄色光線下に於て多くなり、暗黒、青色、紫色之に次ぐに至るも、白色光線下に於ては、終始其生成少なく、其他の光線に於ては48時と96時との生成曲線正反對を示すを見る。
S. ellipsoideus にありては、赤色光線及暗黒下に於て優勢を示し、青色之に次ぎ、紫色及綠色更に之に次げるも黄色及白色光線下に於ては劣勢にして、特に黄色光線は最少を繼續する點 aldehyde の場合と殆んど相似

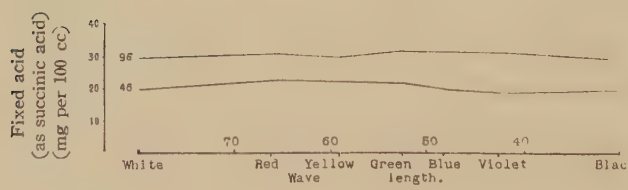
たり。

兩者を通じて揮發酸の生成最多きは青色 I 光線下にありて、其生成に有利なるは波長 4000—5000 I. Å. なるべし。白色光線下に於ける揮發酸の生成は、暗黒下に於けるよりも常に少なく、此點 Lubimenco et Troloff-Bagreief の得たる結果と相反せり (Fig.5, Fig.6)。

5. Fixed acid (as succinic acid) (mg per 100 cc)

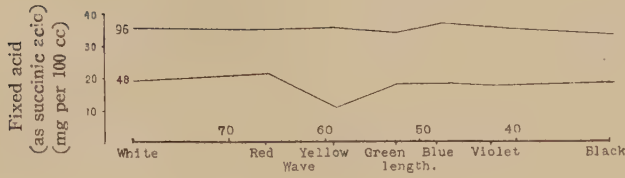
Name of yeast Lapsed time Name of filter	<i>S. cerevisiae</i>		<i>S. ellipsoideus</i>	
	48	96	48	96
White	19.865	29.435	19.117	35.589
Red	23.348	31.357	21.384	35.022
Yellow	23.124	30.678	10.881	35.287
Green	I	20.855	30.224	16.170
	II	24.859	35.362	19.646
	Average	22.857	32.793	17.908
Blue	I	21.610	30.224	16.699
	II	20.477	34.682	19.797
	Average	21.044	32.453	18.248
Violet	I	22.215	32.192	17.303
	II	18.664	32.491	17.039
	Average	20.440	32.342	17.171
Black	21.157	30.640	18.097	32.869

Fig. 7. *S. cerevisiae*



之に依れば、不揮發酸の生成多きは *S. cerevisiae* にありては、初め赤色緑色の光線なれども、後には緑色、青色、紫色の光線となり、白色光線に於ては、暗黒よりも常に其生成少なし。*S. ellipsoi-*

Fig. 8. *S. ellipsoideus*



deus にありては、初めの生成量赤色光線に於て多く、白色、青色及暗黒之に次げるも、後には青色光線に於て優勢となり、白色、黄色、紫色

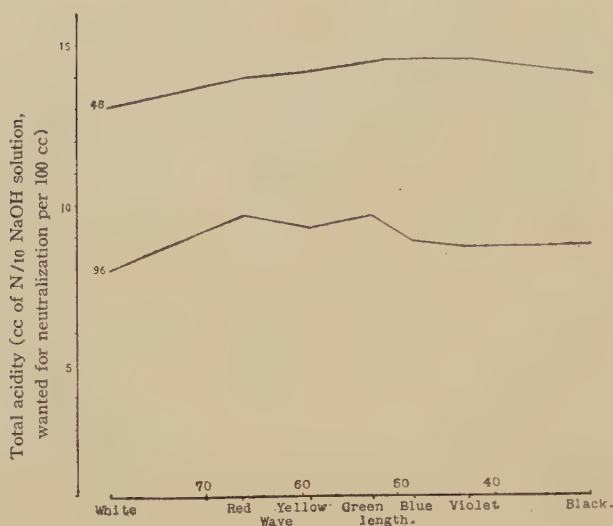
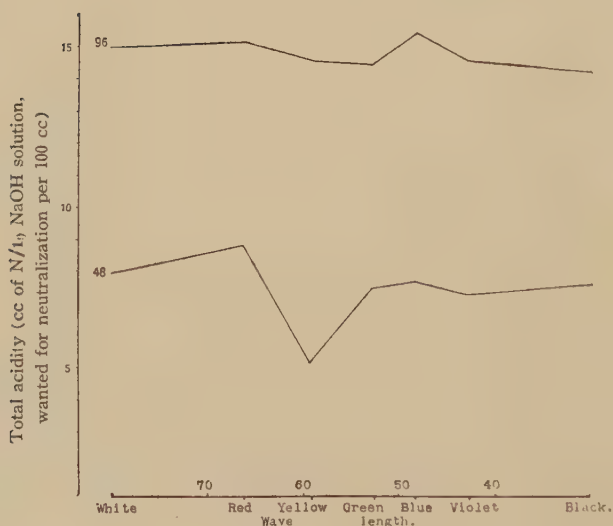
光線之に次ぎ、白色光線にては暗黒よりも常に其生成量多し。

兩者を通じて觀るに、不揮發酸の集積は概して波長 4000 — 5000 I. Å. なる紫色乃至綠色の部に於て多きを知る (Fig.7, Fig.8)。

6. Total acidity (cc of $N/_{10}$ NaOH wanted for neutralization per 100 cc)

Name of yeast		<i>S. cerevisiae</i>		<i>S. ellipsoideus</i>	
Name of filter	Lapsed time	48	96	48	96
White		7.673	12.709	7.688	14.763
Red		9.520	13.662	8.633	14.993
Yellow		9.050	13.845	4.963	14.454
Green	I	9.180	13.550	6.713	13.048
	II	9.735	14.645	7.863	15.647
	Average	9.458	14.098	7.288	14.348
Blue	I	9.430	13.890	7.120	16.272
	II	7.895	14.418	7.993	14.472
	Average	8.663	14.154	7.557	15.372
Violet	I	9.050	14.067	7.088	14.038
	II	7.875	14.071	7.177	15.022
	Average	8.463	14.069	7.133	14.530
Black		8.595	13.537	7.527	14.276

之に依れば、總酸は *S. cerevisiae* にありては、初め綠色及赤色光線下に於て大なりしも、後には青色、綠色及紫色の光線下に於て大となり、白色光線下に於ては終始最小を

Fig. 9. *S. cerevisiae*Fig. 10. *S. ellipsoideus*

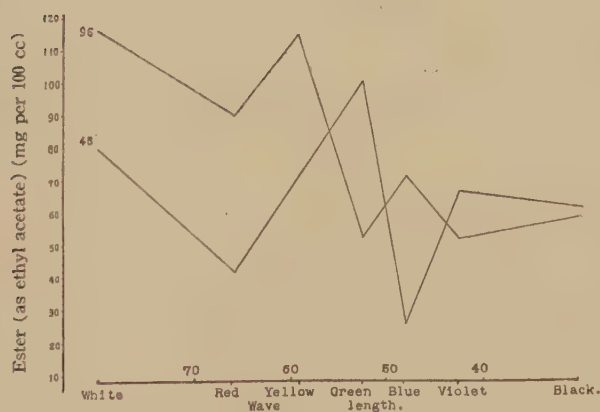
現はし、*S. ellipsoideus* にありては、初め赤色、白色、青色及綠色の光線下に於て大なりしも、後には青色 I, 綠色 II, 紫色 II の光線下に於て大となり、白色光線下に於ける酸度は暗黒下の夫れよりも常に大なるを見たり。兩者を通じて總酸は 4000 — 5000 I. Å.

なる紫色乃至綠色の部に於て集積多きを知るべし。尙ほ表には表はれざりしも醗酵開始後酸の生成は漸次増加しては衰へ又増加し、恰も波狀を描くが如し (Fig. 9, Fig. 10)。

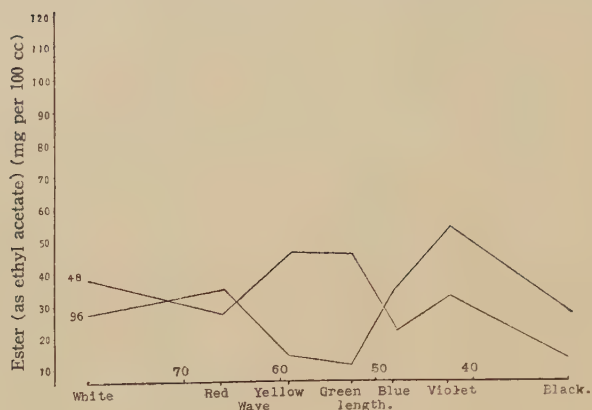
7. Ester (as ethyl acetate) (mg per 100 cc)

Name of yeast Lapsed time Name of filter	<i>S. cerevisiae</i>		<i>S. ellipsoideus</i>	
	48	96	48	96
White	80.432	116.332	38.522	27.736
Red	41.624	89.789	28.006	35.722
Yellow	70.664	115.126	47.322	15.449
Green	I	140.448	57.352	65.802
	II	59.972	49.254	28.710
	Average	100.210	52.303	47.256
Blue	I	0	98.020	32.956
	II	52.844	43.798	12.650
	Average	25.366	70.959	22.803
Violet	I	66.792	42.689	30.690
	II	65.912	60.672	37.301
	Average	66.352	51.681	33.996
Black	61.996	59.246	14.881	28.952

Fig. 11. *S. cerevisiae*



之に依れば, ester
の生成は極めて不規
則なるのみならず,
48時に於て生成量多
きもの96時に至りて
其量を減じ反之前に
生成少なきものは,
後に其量を増すが如
き反對の現象を呈す
るもの多きは, 酸と
alcohol との化合分

Fig. 12. *S. ellipsoideus*

解寸時も休止することなく行はれつつあるを證するものにして、光線との關係は俄に斷定する能はず (Fig.11, Fig.12)。

摘 要

上述の結果を摘要すれば次の如し：—

1. Alcohol の生成は波長 6130 I. Å. 以上の赤色光線下に於て最多く、黄色光線 (6130—5600 I. Å.), 綠色光線 (5600—4890 I. Å.) 之に次ぐも、青色光線 (5600—4500 I. Å.), 紫色光線 (4690—3800 I. Å.), 暗黒下に於ては其生成少なきが故に、alcohol の生成には波長の長き光線有利なりと謂ふべし。

2. Aldehyde の生成は 4000—5600 I. Å. なる紫色乃至帶黃綠色部に於て多き傾あり。

3. Acetal の生成は不規則なり。

4. 揮發酸、不揮發酸の集積は 4000—5000 I. Å. なる紫色乃至綠色部に於て多し。

5. Ester の生成は極めて不規則なり。

6. 醱酵生成物曲線に於て、實驗開始後 48 時と 96 時とに

S. cerevisiae

S. ellipsoideus

alcoholは

赤乃至紫間

黄乃至黒間

aldehyde は	赤乃至黒間	
揮發酸は	赤乃至黒間	
不揮發酸は	赤乃至黒間	
esterは	黄乃至黒間	白乃至黒間

に略對稱的關係を現はせり。

要之、長波長の光線下に於ては alcohol の生成多く、波長之より短くなれば aldehyde の生成を増し、更に短波長となれば酸の量を増す傾あり。此傾向は短波長下に於ては酸の aldehyde への還元、aldehyde の alcohol への還元、長波長下に於けるよりも弱きに因ると信すべき理由あり。

著者の前實驗(4)(5)及現實験を綜合すれば醗酵度高き光線に於ては alcohol の生成量多く、其低き光線に於ては概して aldehyde、酸の集積多きが故に醗酵度の高低と生成物の量との間には相關現象ありと思推せらる。

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Explanation of Plate II

Spectra showing transmission wave length.

Résumé

There are many reports on the influence of ultra-violet rays upon the yeasts and their fermentation. But so far as the author is aware, there is none on the effect of the monochromatic lights upon the fermentation products of yeasts, except that Purvis and Warwick (1906, 1908) have published in regard to the acids produced after fermentation under such light. On the other hand, Lubimenco and Troloff-Bagreief (1912) have determined the quantities of alcohol, carbon dioxide, volatile acids and glycerin, etc. produced under the ordinary light and darkness.

In order to investigate the effect of spectral monochromatic lights on the fermentation products, the author has inoculated 0.0025 g of *Saccharomyces cerevisiae* and of *Saccharomyces ellipsoideus* of the dried yeasts into the test tubes containing 5 cc of Koji extract which were previously sterilized by the ordinary method. The tubes were placed within the tinned boxes, both the inner and outer surfaces of which were japanned black. The boxes were provided with the windows in the front where the light screens were placed. These boxes with their contents were placed in an incubator at 25° C, and an electric lamp was placed opposite the glass door of the incubator at a distance of 175 cm from the front of the boxes. So that the light issued through the glass door and the light screens and then played upon the tubes.

The screens at the front windows of the tinned boxes were made of two glass plates, between which different coloured filters made with gelatine solutions containing various pigments were held. These screens were spectroscopically examined and it was found that they respectively transmitted the light of the wave lengths shown in the following table. They admitted also the transmission of infra-red rays, but no ultra-violet rays.

Table showing the qualities of the filters used.

Name of filter	Transmission wave length (I. Å.)	Colour of transmission rays
White	All visible rays	White
Red	6130 up	Red
Yellow	6700 - (6130 - 5600) - 4900	Red - (Reddish-yellow - Greenish yellow) - Greenish-blue
Green I	5660 - 5040	Yellowish-green - Green
Green II	5460 - 4890	Yellowish-green - Bluish-green
Blue I	5050 - 4730	Green - Violet-blue
Blue II	4960 - 4530	Greenish-blue - Violet-blue

Violet I	4690—3820	Bluish-violet—Violet
Violet II	4410—3960	Violet
Black	None	Dark

The tubes were taken out from each box at the intervals of 48 and 96 hours after the incubation, the contents were distilled, and the determinations of the quantities of alcohol, aldehyde, acetal, volatile acid and ester were conducted from the distillates, and that of the fixed acid from the residual liquids.

In this experiment, the amount of alcohol was always greatest at 6130 I. Å., the red region, and this was followed by that at 6130—4900 I. Å., the red — green regions. The amounts at violet — blue regions were smaller. Thus, the beneficial effect on the production of alcohol is found under the rays of long wave length, and the darkness is very disadvantageous, which is contrary to the result obtained by Lubimenco and Troloff-Bagreief.

The amount of aldehyde was inclined to be great at 4000 — 5600 I. Å. So, the advantageous rays for its production appear to be those in regions of comparatively short wave length. The production of acetal was poor and irregular.

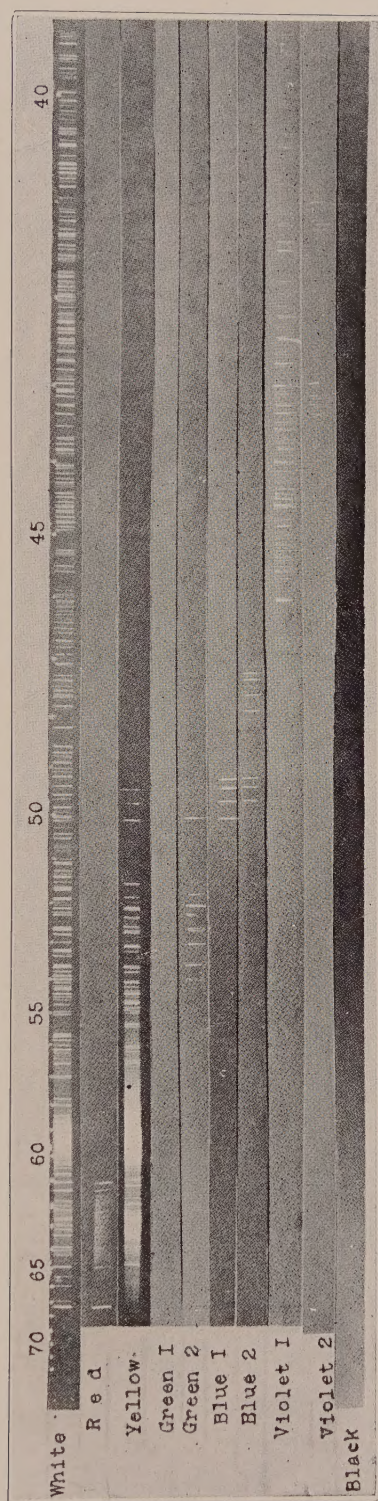
The beneficial rays for the production of volatile and fixed acids were of the regions of 4000 — 5000 I. Å. The amount of volatile acids under the white rays was lower in comparison with that under the darkness, which is also contrary to Lubimenco and Treloff-Bagreief's finding.

The production of ester was very irregular, hence the influence of monochromatic lights on ester in fermenting solutions could not be decided.

The light did not have any remarkable influence on the constituents of the medium during this experimental period.

According to the present experiments and also the author's previous works (1932, a, b) the amount of alcohol increased much under the rays resulting in the higher degree of fermentation. On the other hand, under the rays effecting the lower degree of fermentation, the accumulation of aldehyde and acids was great.

Considering these facts, it may be said that the reduction is vigorous under the rays of long wave length. So that such rays are more profitable for the production of alcohol than the rays of comparatively short wave length such as violet — green, which are advantageous in the accumulation of aldehyde and acids.



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